National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 955c

Toxic Metals in Caprine Blood

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of lead, arsenic, cadmium, mercury (total), ethylmercury, inorganic mercury, and methylmercury concentration determinations in whole blood. It can also be used in validation schemes for analytical methods and in traceability schemes for working or secondary blood reference materials containing these constituents. A unit of SRM 955c consists of four vials of frozen caprine blood at four concentration levels: a base level and three progressively elevated levels that contain endogenous lead and spiked inorganic arsenic, cadmium, ethylmercury, inorganic mercury, and methylmercury. Each vial contains approximately 2 mL of whole blood.

Certified Concentration Values: Certified concentration values for selected toxic elements and species in SRM 955c are provided in Tables 1 through 4. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values are based on the mean results from a single NIST primary method. The uncertainties associated with each certified value are expressed as expanded uncertainties with a confidence level of approximately 95 % [2].

Reference Concentration and Density Values: Reference concentration values for selected toxic elements in SRM 955c are also provided in Tables 1 through 4. Reference density values for Levels 1 through 4 are provided in Table 5. A reference value is a noncertified value that is the best estimate of the true value based on available data [1]. These values do not meet NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference values are based on the mean results from a single NIST method for arsenic in Level 1 and Level 3 and on results from collaborating laboratories for arsenic, cadmium, and total mercury in Level 2 and Level 4. The uncertainties associated with each reference value are expressed as expanded uncertainties with a confidence level of approximately 95 % [2].

Expiration of Certification: The certification of **SRM 955c** is valid, within the measurement uncertainty specified, until **01 February 2020**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of measurements leading to the certification of this material were performed by K.E. Murphy and G.C. Turk of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by D.P. Berry, C.E. Bryan, W.C. Davis, S.E. Long, J.L. Molloy, K.E. Murphy, and T.W. Vetter of the NIST Chemical Sciences Division.

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Experimental design and statistical analysis of the data were provided by W.F. Guthrie of the NIST Statistical Engineering Division.

Collection, preparation, and homogeneity assessment for lead, cadmium, and mercury were performed by P.J. Parsons, C. Geraghty, C.D. Palmer, and M.E. Lewis, Jr. of the Division of Environmental Health Sciences, Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health (NYSDOH; Albany, NY). Additional data for lead, cadmium, mercury, and arsenic were provided by P.J. Parsons from reference laboratories participating in the New York State Department of Health proficiency testing (PT) program for blood lead and trace elements.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS[(1)]

SRM 955c IS INTENDED FOR RESEARCH USE. This SRM is derived from whole caprine (goat) blood collected at NYSDOH's Wadsworth Center according to a protocol approved by the Center's Institutional Animal Care and Use Committee. The Wadsworth Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, has an approved Animal Welfare Assurance (#A3183-01) with the Public Health Service, and is registered as a Class R Research Facility (#21-R-0124) with the United States Department of Agriculture. The Wadsworth Center affirms that the donor animals were sourced in the United States.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The blood is shipped frozen (on dry ice) and, upon receipt, must be stored frozen until ready for use. The SRM should be kept in its original vials and stored frozen at or below –20 °C. The vials should be stored in the box and aluminized bag in which they are supplied. Frost-free freezers should not be used because of temperature fluctuations.

Use: Before use, a frozen sample should be allowed to thaw at room temperature. The sample should be mixed by gently rocking or mildly swirling (not shaking) the vial to remix any water that may have separated on freezing. Shaking will cause bubbles to form at the top of the sample. Do not use if clotted. The contents of a vial may be thawed, a sample withdrawn, and the contents refrozen. Because of possible evaporative losses, it is advised that the contents of a vial not be used if less than one-third of the original blood volume remains. For the certified concentration to be applicable to an analytical determination, a minimum sample of 200 µL must be used.

SOURCE, PREPARATION, AND ANALYSIS

Source: The source of blood for this SRM was goats that had been dosed with gelatin capsules containing lead acetate at the Wadsworth Center's Griffin Laboratory (Guilderland, NY) according to a standard protocol established for blood lead proficiency testing purposes [3]. Each unit of blood was collected into a sterile blood bag containing dipotassium ethylene diamine tetraacetic acid (EDTA) at a concentration of 1.5 mg/mL as an anticoagulant.

Preparation: At the Wadsworth Center, individual blood units were analyzed for lead by graphite furnace atomic absorption spectrometry (GFAAS) [4], filtered through cheesecloth, and then blended under Class 100 clean room conditions into a single pool at the desired lead concentration. This procedure was repeated to produce four pools at different lead concentrations. Levels 2 through 4 were further supplemented with inorganic arsenic, cadmium, and mercury as ethylmercury, inorganic mercury, and methylmercury. Blood (2 mL) was dispensed into 3.8 mL high-density polyethylene vials, which were capped and stored at –80 °C. The fill sequence was preserved. The frozen pools were shipped overnight to NIST on dry ice.

Homogeneity Assessment: To test for homogeneity, 8 to 12 vials per level and method were selected based on a randomized sampling plan. Lead, cadmium, and mercury (total) in the four levels were measured in duplicate 200 µL portions by inductively coupled plasma mass spectrometry (ICP-MS) [5,6]. GFAAS was used to measure lead in Levels 2, 3, and 4 in duplicate 50 µL portions [4,6]. A LECO AMA 254 mercury analyzer was used to measure mercury (total) in duplicate 40 mg portions in Level 3. Arsenic was measured in Levels 1 and 3 in duplicate 1 g portions by ICP-MS. Based on statistical analysis of the analytical results by NIST, an allowance for potential uncertainty from material heterogeneity was added for lead in Level 1, cadmium in Levels 1 and 2, and mercury (total)

[(1)] Certain commercial equipment, instruments, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

in Levels 1, 2, 3, and 4. Because mercury (total) was found to exhibit heterogeneity, an allowance for material heterogeneity was added for ethylmercury, inorganic mercury, and methylmercury in Level 3.

Determination of Lead at NIST (Levels 1, 2, 3, and 4): Lead was measured using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) [6,7]. A single portion from nine vials at each concentration level, selected based on a randomized sampling plan, were analyzed. For Level 1, the entire content of each vial was analyzed. For Levels 2 through 4, 1 g portions were analyzed. Isotopically enriched ^{206}Pb was added to the test portions prior to digestion with nitric acid in a high-pressure microwave system. For Level 1, test portions were additionally wet-ashed with 0.7 g of perchloric acid. The residue was redissolved in 2 % volume fraction nitric acid prior to measurement.

Determination of Cadmium at NIST (Levels 1 and 3): Cadmium was measured using ID-ICP-MS [8]. For Level 1, eleven test portions were analyzed with sample sizes ranging from the entire content of each vial (1.8 g) to the combined content of two vials (3.6 g). For Level 3, the entire content of each of six vials was analyzed. Isotopically enriched ^{111}Cd was added to the test portions prior to digestion with nitric acid in a high-pressure microwave system. Samples were subjected to anion exchange chromatography prior to analysis to reduce spectral and non-spectral interference.

Determination of Mercury (Total) at NIST (Levels 1 and 3): Mercury (total) was measured using ID-ICP-MS with cold-vapor mercury generation using tin [II] chloride reductant [9]. For Level 1, single 1 g portions from five vials were analyzed. For Level 3, duplicate 0.2 g portions from five vials were analyzed. Isotopically enriched ^{201}Hg was added to the test portions prior to digestion with nitric acid in a high-pressure microwave system. Following digestion, samples were diluted with quartz-distilled water. Samples were allowed to degas overnight at 4 °C, prior to further dilution and measurement.

Determination of Ethylmercury, Inorganic Mercury, and Methylmercury at NIST (Level 3): Ethylmercury, inorganic mercury, and methylmercury were measured using triple-spike speciated isotope dilution gas chromatography (GC) ICP-MS. There were no detectable organomercury species in Level 1, and the content of inorganic mercury in Level 1 was below the current method detection limit. For Level 3, single 0.5 g portions from six vials were analyzed. Isotopically enriched $CH_3{}^{202}Hg$, $C_2H_5{}^{201}Hg$, and ^{198}Hg were added to test portions prior to extraction with 25 % mass fraction tetramethylammonium hydroxide in water using a focused microwave system. Samples were buffered at pH 5, derivatized with 20 % mass fraction sodium tetrapropylborate in water, back-extracted into hexane, and subjected to clean up on solid-phase extraction cartridges packed with 5 % mass fraction water-deactivated alumina. Collected fractions were concentrated to 0.2 mL and analyzed by GC/ICP-MS.

NIST Analyses for Arsenic (Levels 1 and 3): Arsenic was measured using standard additions collision cell technology (CCT) ICP-MS. Duplicate 1 g portions from eight vials of each of Level 1 and Level 3 were spiked with an arsenic standard or dummy solution and ruthenium and rhodium internal standards prior to digestion with nitric acid and peroxide in a focused microwave system. Samples were diluted in 1 % volume fraction butanol in water prior to analysis by CCT-ICP-MS in kinetic energy discrimination mode using 8 % mole fraction H_2 in balance (92 %) mole fraction He.

Proficiency Testing Analyses for Cadmium, Mercury (total), and Arsenic (Levels 2 and 4): Blind samples of SRM 955c were distributed for analysis in addition to regularly scheduled PT samples as part of a special education event conducted by NYSDOH. Samples were analyzed in the same manner as routine patient specimens. A subset of the reported data, composed of results from a group of experienced reference laboratories as specified by NYSDOH, was used to report values for Levels 2 and 4. For cadmium results from 22 laboratories employing GFAAS (3), AAS (1), ICP-MS (16), DRC/ICP-MS (1), and HR-ICP-MS (1) were used. For mercury results from 12 laboratories employing ICP-MS (10), CV-AAS (1), and HR-ICP-MS (1) were used. For arsenic results from 9 to 10 laboratories employing ICP-MS (8 to 9) and HR-ICP-MS (1) were used. The equally weighted means from each reference laboratory were used to calculate the assigned reference values.

Table 1. Concentration Values for SRM 955c Caprine Blood, Level 1

Constituent	Units	Certified Value[a]	Reference Value[b]	ν_{eff}	k
Lead	µg/dL	0.424 ± 0.011		42.8	2.02
Arsenic	µg/L		2.07 ± 0.63	14.6	2.14
Cadmium	µg/L	0.0317 ± 0.0062		12.7	2.17
Mercury (Total)	µg/L	0.017 ± 0.011		≥60	2.00

Table 2. Concentration Values for SRM 955c Caprine Blood, Level 2

Constituent	Units	Certified Value[a]	Reference Value[b]	ν_{eff}	k
Lead	µg/dL	13.950 ± 0.080		≥60	2.00
Arsenic	µg/L		21.9 ± 1.7	8.0	2.31
Cadmium	µg/L		2.14 ± 0.24	18.3	2.10
Mercury (Total)	µg/L		4.95 ± 0.76	20.3	2.08

Table 3. Concentration Values for SRM 955c Caprine Blood, Level 3

Constituent	Units	Certified Value[a]	Reference Value[b]	ν_{eff}	k
Lead	µg/dL	27.76 ± 0.16		≥60	2.00
Arsenic	µg/L		53.9 ± 3.4	8.1	2.30
Cadmium	µg/L	5.201 ± 0.038		≥60	2.00
Mercury (Total)	µg/L	17.8 ± 1.6		5.2	2.54
Ethylmercury (as Hg)	µg/L	5.06 ± 0.47		5.9	2.46
Inorganic Mercury (as Hg)	µg/L	9.0 ± 1.3		5.8	2.47
Methylmercury (as Hg)	µg/L	4.5 ± 1.0		5.5	2.50

Table 4. Concentration Values for SRM 955c Caprine Blood, Level 4

Constituent	Units	Certified Value[a]	Reference Value[b]	ν_{eff}	k
Lead	µg/dL	45.53 ± 0.27		≥60	2.00
Arsenic	µg/L		77.5 ± 4.2	9.0	2.26
Cadmium	µg/L		9.85 ± 0.17	21.0	2.08
Mercury (Total)	µg/L		33.9 ± 2.1	18.1	2.10

[a] The uncertainty in each certified value is given as an expanded uncertainty, $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and k is a coverage factor used to obtain an approximate level of confidence of 95 %. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of uncertainty components associated with random measurement error, systematic sources of measurement error, and material heterogeneity, where applicable. The value of k is determined from Student's t distribution with ν_{eff} effective degrees of freedom. The measurands are the certified concentrations of the constituents listed in Tables 1 through 4. Metrological traceability is to the SI derived unit for concentration (expressed as micrograms per deciliter or micrograms per liter).

[b] The uncertainty in each reference value is given as an expanded uncertainty, $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and k is a coverage factor used to obtain an approximate level of confidence of 95 %. For arsenic in Level 1 and Level 3, the value of u_c is intended to represent, at the level of one standard deviation, the combined effect of uncertainty components associated with random measurement error and systematic sources of measurement error. For arsenic, cadmium, and mercury (total) in Levels 2 and 4, the value of u_c is intended to represent the combined effect of uncertainty components associated with random measurement errors within and between laboratories, and material heterogeneity, where applicable. The value of k is determined from Student's t distribution with ν_{eff} effective degrees of freedom. The measurands are the reference concentrations of the constituents listed in Tables 1 through 4 as determined by the methods stated above. Metrological traceability is to the SI derived unit for concentration (expressed as micrograms per deciliter or micrograms per liter).

Table 5. Reference Density Values for SRM 955c, Levels 1 through 4[(a)]

Level	Units	Density at 22 °C		
Level 1	g/mL	1.05182	±	0.00091
Level 2	g/mL	1.05277	±	0.00058
Level 3	g/mL	1.05292	±	0.00069
Level 4	g/mL	1.05265	±	0.00065

[(a)] Densities were determined at NIST using a semi-micro gravimetric method [10] and are provided to allow conversions between results expressed as mass concentrations and mass fractions. The uncertainty in each reference value is given as an expanded uncertainty, $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and $k = 2$ is a coverage factor used to obtain an approximate level of confidence of 95 %. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of uncertainty components associated with random measurement error and systematic sources of measurement error. The measurands are the reference concentrations of the densities listed in Table5 as determined by the method stated above. Metrological traceability is to the SI derived unit for concentration (expressed as grams per milliliter).

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Certificate Revision History: **27 January 2016** (Editorial changes); **28 July 2010** (Change of cadmium and total mercury information concentration values to certified values in Level 1 and Level 3; change of cadmium and total mercury information concentration values to reference values in Level 2 and Level 4; addition of arsenic reference concentration values in Levels 1 through 4; change of information density values to reference values in Levels 1 through 4; addition of ethylmercury, inorganic mercury, and methylmercury certified concentration values in Level 3; change of SRM name to *Toxic Metals in Caprine Blood*; extension of certification period; editorial changes); **05 February 2007** (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.